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Thanks

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Topical Immune Modulation with Dinitrochlorobenzene in HIV Disease: A Controlled Trial from Brazil

Abstract

Objective: Despite the rapid spread of human immunodeficiency virus (HIV) in the developing countries of Africa, Asia and Latin America, accessible and affordable antiretroviral therapies have not been developed. Dinitrochlorobenzene (DNCB) is an inexpensive contact sensitizing agent that stimulates cell-mediated immunity when applied to the skin. We have examined the clinical and immunologic effects of topical DNCB therapy in a cohort of indigent patients with HIV disease from Brazil. **Design and Methods:** Thirty-five HIV-infected subjects were divided into a control group that refused DNCB therapy (6 patients) and a treatment group that applied topical DNCB on a weekly basis throughout the study (29 patients). Subjects were monitored for adverse clinical events, progression to AIDS and changes in body weight. CD4 and CD8 T-cell counts were also monitored in both groups. **Results:** Control and treated patients were evenly matched in terms of age, initial clinical status and prior adverse clinical events. The mean follow-up was 19.7 months for the control group and 17.8 months for the DNCB group. Control patients had significantly more adverse clinical events and progression to AIDS during the study than the treatment group ($p=0.002$ and $p=0.013$, respectively). There were no deaths in either group. Control patient weights decreased over the study period while DNCB patient weights increased ($p<0.001$). CD4 and CD8 T-cell counts decreased significantly in the control group and increased in the DNCB group ($p<0.001$ and $p=0.031$, respectively). DNCB therapy was well tolerated. **Conclusions:** Topical DNCB therapy affords a rational, effective and inexpensive treatment approach for HIV disease. DNCB should benefit patients in developing nations with limited access to health care.

Introduction

In recent years, the acquired immune deficiency syndrome (AIDS) has spread rapidly in the developing countries of Africa, Asia and Latin America [1, 2]. It is esti-

mated that by the year 2000, as many as 40 million people with human immunodeficiency virus (HIV) infection, or 90% of the world's cases, will be found in these countries [3]. Efforts to control the spread of AIDS in developing nations have focused primarily on prevention of HIV trans-

Table 1. Clinical characteristics of study subjects

Subjects	n	M/F	Mean age ± SD, years	On study ± SD, months	Initial clinical status		Adverse clinical events		Progression to AIDS
					asympt./ARC	AIDS	prior to study	during study	
Control	6	4/2	34.3 ± 5.8	19.7 ± 2.8	5/6 (83)	1/6 (17)	3/6 (50)	6/6 (100)*	2/5 (40)**
DNCB	29	15/14	37.0 ± 2.4	17.8 ± 1.3	25/29 (86)	4/29 (14)	12/29 (41)	6/29 (21)*	1/25 (4)**

n = Number of patients; M/F = male/female ratio; SD = standard deviation; asympt. = asymptomatic; ARC = AIDS-related complex.
*p=0.002, **p=0.013. Figures in parentheses indicate percentages.

mission, since antiretroviral chemotherapy is financially unfeasible for these countries [1-3]. Development of affordable HIV therapies for the bulk of the world's population remains a significant priority, and little progress has been made in this area.

Dinitrochlorobenzene (DNCB) is a simple contact sensitizing agent related to poison ivy and poison oak that has been used in color photography for over 50 years [4]. DNCB is also a potent stimulant of the cellular immune system when applied to the skin. Topical DNCB therapy has been associated with improved clinical and immunologic parameters in HIV-infected patients when the compound is used on a regular basis over a number of years [5-8]. The cost of this therapy is about US\$ 0.30 (30 cents) per week.

We undertook a controlled trial of topical DNCB therapy in a cohort of indigent patients from Brazil, a country with limited access to HIV therapies [9-13].

Patients and Methods

The study was performed at a single public hospital in Porto Alegre where patients received free medical care. Inclusion criteria were as follows: (1) HIV seropositivity with CD4 T-cell counts of 100-600/ μ l and (2) no prior exposure to DNCB. Thirty-five patients (19 men and 16 women) were enrolled in the trial after informed consent had been obtained. The clinical characteristics of the study subjects are shown in table 1. Risk factors for HIV disease included homosexual contact (15 patients), intravenous drug use (9 patients) and heterosexual contact (11 patients). Six patients underwent an initial topical DNCB application but then refused further therapy for cosmetic reasons. These patients continued to be monitored clinically and served as the control group. The remaining 29 patients had weekly applications of topical DNCB throughout the course of the study.

Topical DNCB therapy was carried out as previously described [5], but with several modifications. DNCB in crystal form (>98% pure) was obtained from Kodak Chemicals SA, São Paulo, and dissolved in reagent-grade acetone. Patients were sensitized with a 10% solution of DNCB applied to a 3-cm-square area of skin. Two weeks

Table 2. Infectious complications during the study

Infection	Control subjects (n = 6)		DNCB subjects (n = 29)	
	prior to study	during study	prior to study	during study
Fungus ¹				
Oral	0	1 (17)	0	0
Pulmonary	0	0	0	1 (3)
Vaginal	0	0	0	2 (7)
Bacteria				
Skin ²	0	1 (17)	0	0
Pulmonary ³	0	0	1 (3)	0
Intestinal parasites ⁴	2 (33)	1 (17)	7 (24)	1 (3)
Syphilis	1 (17)	0	3 (10)	0
Herpes zoster	0	2 (33)	0	2 (7)
Pulmonary tuberculosis	0	0	1 (3)	0
<i>Pneumocystis carinii</i> pneumonia	0	1 (17)	0	0
Total	3 (50)	6 (100)	12 (41)	6 (21)

Figures in parentheses indicate percentages.

n = Number of patients.

¹ *Candida* species.

² *Staphylococcus aureus*.

³ *Klebsiella*.

⁴ *Ascaris* (1), *Entamoeba* (4), *Giardia* (1), *Strongyloides* (2), *Taenia* (2), unclassified (1).

Table 3. Mean body weight changes in study subjects

Subjects	n	Mean initial weight, kg ± SD	Mean follow-up weight, kg ± SD	Mean change kg ± SD
Control	6	63.8 ± 8.4	60.3 ± 6.6	-3.5 ± 4.4**
DNCB	29	62.0 ± 8.0*	64.9 ± 9.0*	+2.9 ± 2.6**

n = Number of patients; SD = standard deviation. *p<0.001.
**p<0.001.

Table 4. Mean CD4 and CD8 T-cell counts in study subjects

Subjects	n	Initial		Follow-up		Mean change	
		CD4 T cells	CD8 T cells	CD4 T cells	CD8 T cells	CD4 T cells	CD8 T cells
Control	6	317 ± 105 ^a	902 ± 286 ^b	147 ± 55 ^a	546 ± 194 ^b	-170 ± 94 ^d	-356 ± 195 ^c
DNCB	29	351 ± 160 ^a	865 ± 460	397 ± 166 ^c	1,065 ± 651	+46 ± 111 ^d	+200 ± 591 ^c

T-cell numbers represent cells/ μ l. n = Number of patients; SD = standard deviation.
^ap = 0.007, ^bp = 0.007, ^cp = 0.035, ^dp < 0.001, ^ep = 0.031.

after the initial sensitization, therapy was resumed with the application of 2% DNCB solution to a different 3-cm-square area of skin. The 2% DNCB treatment was continued on a weekly basis throughout the study. However, starting at 2 months after the initiation of therapy, each patient received a 10% DNCB booster application once a month instead of the regular 2% application. Excessive skin reactions were treated with cold compresses soaked in camomile tea. All DNCB applications were performed by a single investigator (A.T.) in a standardized fashion using rotating areas of skin on the trunk and extremities.

Patients had blood tests, body weight measurement and fecal parasitic examination performed approximately every 3 months. Blood tests included complete blood count, Venereal Disease Research Laboratory test for syphilis and lymphocyte subset analysis. Analysis of CD4 and CD8 T cells was performed by one central laboratory using flow cytometry, as previously described [5]. To ensure consistency, baseline and follow-up T-cell studies were performed at a time when patients were not acutely ill. Viral load testing was not available at the time of the study.

Antiretroviral chemotherapy and prophylactic antibiotics were not obtainable by the study subjects. Patients were treated for complications of HIV disease as the need arose. Fungal infections were treated with topical or systemic ketoconazole. Syphilis was treated with high-dose penicillin, and parasitic disease was treated with thiabendazole. Patients with herpesvirus infections received acyclovir, and *Pneumocystis carinii* pneumonia was treated with oral trimethoprim/sulfamethoxazole. Pulmonary tuberculosis was treated with isoniazid and streptomycin. No other medications were used in the study.

Statistical analysis was performed using the paired Student *t* test for intragroup comparisons and the unpaired Student *t* test for intergroup evaluations. The *t* test was used because the analysis involved noncategorical data and the variance of the population was unknown [14].

Results

The clinical and immunologic outcomes of the study are shown in tables 1-4. As shown in table 1, the control and DNCB treatment groups were evenly matched in terms of age and initial clinical status. The incidence of adverse clinical events prior to the study was also similar for the two groups (50 vs. 41%, p = n.s.). There was a relatively high incidence of parasitic disease (9/35 patients, 26%) and syph-

ilis (4/35 patients, 11%) prior to the start of the trial. The mean follow-up was similar for the control and DNCB groups (19.7 vs. 17.8 months, p = n.s.). During the study, each of the control patients experienced an adverse clinical event compared to 6/29 DNCB-treated patients (100 vs. 21%, p = 0.002). Over the course of the study, 2/5 control patients progressed to AIDS, while only 1/25 treated patients had disease progression (40 vs. 4%, p = 0.013). Adverse clinical events included fungal, bacterial, parasitic and herpetic infections, as shown in table 2. There were no deaths in either group.

Changes in patient body weights are shown in table 3. Initial weights were comparable in the control and treatment groups. The mean weight decreased by 3.5 kg in the control group over the study period (p = n.s.). In contrast, the mean weight increased by 2.9 kg in the treatment group (p < 0.001). The difference in mean weight change between the two groups was highly significant (p < 0.001).

Changes in mean CD4 and CD8 T-cell counts are shown in table 4. Initial CD4 and CD8 T-cell levels were comparable in the control and treatment groups. In the control group, both the CD4 and the CD8 T-cell counts decreased significantly over the course of the study (p = 0.007 for both subsets). In contrast, CD4 T cells increased significantly in the DNCB-treated patients (p = 0.035). Although CD8 T-cells also increased in these patients, the difference was not statistically significant. However, the change in mean T-cell counts in the control group compared to the treatment group was significant for both CD4 T cells (p < 0.001) and CD8 T cells (p = 0.031) over the course of the study.

DNCB applications were well tolerated, and none of the DNCB-treated patients discontinued topical therapy. Excessive skin reactions were effectively controlled with the cold camomile compresses, and no infections were seen as a result of these reactions.

Discussion

The current study represents the first controlled trial of topical DNCB therapy in HIV disease. Patients treated with DNCB had a significantly lower incidence of adverse clinical events and significantly less progression to AIDS compared to controls. They also had significant weight gain and increased T-cell counts compared to the control subjects. These findings confirm previous reports of beneficial clinical and immunologic effects of topical DNCB therapy in HIV disease [5-8]. The study also confirmed the safety of standardized DNCB application over a prolonged period of time [15, 16].

Several features of the current study differ significantly from previous DNCB trials [5-8]. First, the current study included the largest group of women ever treated with topical DNCB. This aspect is particularly important for future use of DNCB in developing countries where the majority of HIV-infected patients are women. Second, all topical DNCB applications were performed by a single investigator, thereby ensuring a standardized treatment methodology for all subjects. Third, the DNCB protocol used in the study differed from the so-called Epstein protocol described previously [7] in that a monthly 10% DNCB booster dose was used to supplement the weekly 2% DNCB applications. This monthly booster may have enhanced the effect of chronic DNCB therapy. Fourth, confounding antiretroviral chemotherapy was not available to the study subjects. Thus, the present trial represents the broadest, purest and most aggressive DNCB evaluation reported to date. Although our study does not conform to the 'intent-to-treat' model used in larger clinical trials, the study results have validity based on the 'pragmatic' design used in smaller trials [17]. The results require confirmation in a larger group of therapy-naïve HIV patients.

The incidence of HIV disease in Brazil is among the highest in the world [9-13]. The clinical spectrum of op-

portunistic infections in Brazilian HIV patients is similar to that of Africa and Haiti, with high rates of tropical, parasitic and venereal diseases [2, 9]. In addition, the rate of HIV disease progression in Brazil is more rapid than in Europe and North America [9]. In spite of its vast natural resources, Brazil has many of the social, economic and medical limitations of poorer countries coping with HIV disease. In particular, therapeutic options for indigent HIV patients are extremely limited due to the high cost of medications and medical care [9, 10]. For example, a course of combination antiretroviral therapy costs a minimum of US\$ 830.00 per month. This price is out of reach for most Brazilian HIV patients. In contrast, topical DNCB therapy costs about US\$ 1.20 per month. Although even this amount may be excessive for indigent patients, the cost is more feasible than that of standard antiretroviral drugs.

Another advantage of topical DNCB therapy is its ease of use. The weekly application schedule is based on what is known concerning the effect of DNCB on the cellular immune response [18-20]. Topical DNCB induces a delayed-type hypersensitivity skin reaction that in turn stimulates a systemic cellular immune response involving antigen-presenting cells, cytotoxic CD8 T cells and natural killer cells [5, 6]. This cellular immune response has been shown to control HIV replication and disease progression [21-23]. The present study did not evaluate cytotoxic CD8 T-cell subsets, natural killer cells or viral load in DNCB-treated patients. A larger controlled trial of DNCB will examine these immunologic parameters in HIV patients.

In summary, we have performed a standardized, controlled trial of topical DNCB therapy in HIV patients from Brazil. DNCB therapy is associated with improved clinical and immunologic parameters in these patients. The cost of topical DNCB therapy is minimal compared to antiretroviral chemotherapy. DNCB treatment represents a logical and accessible therapeutic approach for developing nations with limited resources to combat the AIDS epidemic.

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